Concise Review: Can the Intrinsic Power of Branching Morphogenesis Be Used for Engineering Epithelial Tissues and Organs?

SANJAY K. NIGAM

Key Words. Kidney • Nephrogenesis • Organ development • Tissue regeneration •
Bioartificial device • Tissue engineering • Bioreactor • Three-dimensional scaffold

Departments of Pediatrics, Medicine, Cellular and Molecular Medicine, and Bioengineering, University of California San Diego, La Jolla, California, USA

Correspondence: Sanjay K. Nigam, M.D., University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0693, USA. Telephone: 858-822-3482; Fax: 858-833-3483; E-Mail: snigam@ucsd.edu

ABSTRACT

Branching morphogenesis is critical to the development of organs such as kidney, lung, mammary gland, prostate, pancreas, and salivary gland. Essentially, an epithelial bud becomes an iterative tip-stalk generator (ITSG) able to form a tree of branching ducts and/or tubules. In different organs, branching morphogenesis is governed by similar sets of genes. Epithelial branching has been recapitulated in vitro (or ex vivo) using three-dimensional cell culture and partial organ culture systems, and several such systems relevant to kidney tissue engineering are discussed here. By adapting systems like these it may be possible to harness the power inherent in the ITSG program to propagate and engineer epithelial tissues and organs. It is also possible to conceive of a universal ITSG capable of propagation that may, by recombination with organ-specific mesenchymal cells, be used for engineering many organ-like tissues similar to the organ from which the mesenchyme cells were derived, or toward which they are differentiated (from stem cells). The three-dimensional (3D) branched epithelial structure could act as a dynamic branching cellular scaffold to establish the architecture for the rest of the tissue. Another strategy—that of recombining propagated organ-specific ITSGs in 3D culture with undifferentiated mesenchymal stem cells—is also worth exploring. If feasible, such engineered tissues may be useful for the ex vivo study of drug toxicity, developmental biology, and physiology in the laboratory. Over the long term, they have potential clinical applications in the general fields of transplantation, regenerative medicine, and bioartificial medical devices to aid in the treatment of chronic kidney disease, diabetes, and other diseases. STEM CELLS TRANSLATIONAL MEDICINE 2013;2:993–1000

INTRODUCTION

A wide variety of tissue engineering strategies are being used in attempts to build organ-like tissues. The perception that it may be possible to replace the function of a complex organ like the failing kidney with a tissue-engineered construct, together with the rapid progress in what is often called regenerative medicine, has helped spur improvements in many technologies, such as cell sourcing, biocompatible scaffolds, bioreactors, three-dimensional (3D) printing, and other methods [1]. Here the focus is on epithelial organs (e.g., kidney, lung, salivary gland, pancreas) consisting in large part of branched ducts and tubules made up of polarized epithelial cells. Although many of the key concepts are generally applicable to most branched epithelial tissues, here they are largely discussed in the context of tissue engineering and development of one organ: the kidney.

USING IN VITRO DEVELOPMENTAL MODELS TO ENGINEER ORGANS: THE EXAMPLE OF THE KIDNEY

Because of the many different morphogenetic processes and cell types involved, the kidney is one of the most complex developing epithelial organs. The metanephric kidney begins as an epithelial bud, known as the ureteric bud (UB), which is an outgrowth of the Wolffian duct (Fig. 1). This process occurs at approximately embryonic day 12 in the rat (and approximately week 4 in the human fetus). Under the influence of soluble factors and cell-cell interactions with the metanephric mesenchyme (MM), the newly formed UB undergoes iterative tip-stalk generation to form the developing collecting duct system—a differentiated tree emerging from multiple rounds of UB branching [2–4]. This collecting duct tree, ultimately consisting of approximately 1 million ductal tips in humans (formed from roughly 20 iterations of branching morphogenesis of the UB), feeds into the ureter. Meanwhile—in a process possibly unique to the kidney mesenchyme—the MM epithelializes and then undergoes tubulogenesis to form the more proximal parts of the nephron (the functional unit of the kidney), including the epithelial portion of the glomerulus (the filtration unit of the nephron), the proximal convoluted tubule, loop
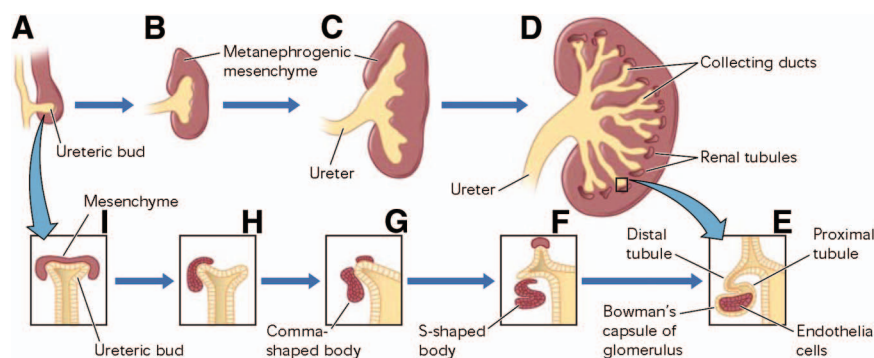
Figure 1. Development of the metanephric kidney. The schematic diagram illustrates the development of the metanephric kidney from its progenitor tissues, the ureteric bud (UB) and the metanephric mesenchyme (MM). **(A):** Metanephric kidney development is initiated with the outgrowth of the UB from the Wolffian duct/pronephric duct and its penetrance into the MM in response to soluble factors (e.g., glial-derived neurotrophic factor) elaborated by this aggregation of intermediate mesoderm-derived cells. **(B–D):** A macroscopic view of kidney collecting system development through UB branching morphogenesis within the MM. **(E–I):** Depiction of nephron development from the MM at the tips of the branching UB. From [2]; used with permission.
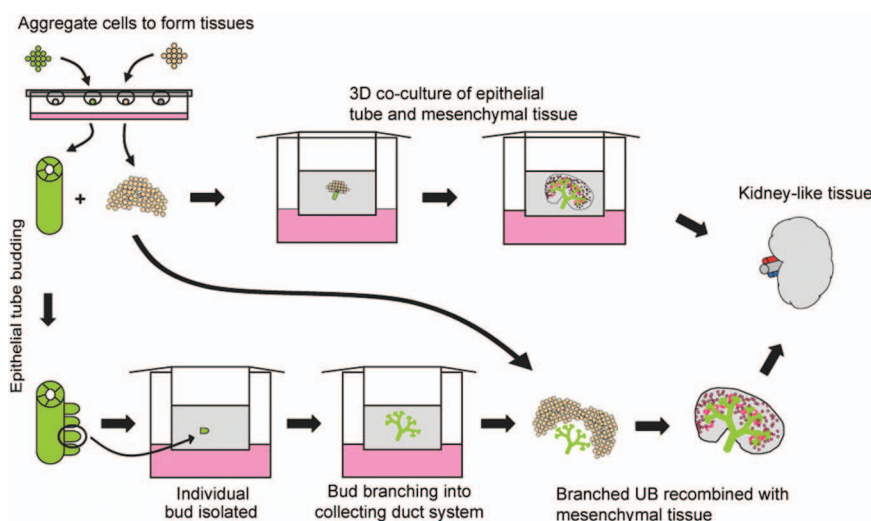

Figure 2. Developmental approaches to in vitro engineering of organ-like tissues. In this strategy, cell lines (either established or primary) from both the epithelial and mesenchymal components of an organ are cultured under conditions that induce their aggregation (i.e., hanging drop cultures). The epithelial cell aggregates are induced to form epithelial tubules that can either be (a) recombined directly with the mesenchymal aggregates in 3D extracellular matrix (ECM) gels leading to the development of an organ-like tissue (e.g., kidney), or (b) induced to form epithelial buds, which can be isolated and cultured in a 3D ECM gel, where the bud will undergo several rounds of branching morphogenesis. Branched epithelial structures are isolated and recombined with mesenchymal aggregates to form an organ-like tissue. In both cases the organ-like tissue (in this instance a kidney-like tissue) is available for implantation/transplantation. From [22]; used with permission. Abbreviations: 3D, three-dimensional; UB, ureteric bud.

of Henle, and the distal tubule [5, 6]. These structures (formed from the MM) are involved in drug or toxin handling, regulation of salt balance, and maintenance of acid-base homeostasis, whereas the collecting ducts (formed from UB branching) play an essential role in water balance [7]. Importantly, kidney development seems to occur in stages that can be defined morphologically [5], by distinct gene expression signatures [8], or by computational analysis of global gene expression patterns [9]. This is important in that there are, apart from functionality, several different measures of differentiation that could be applied to an organ-like structure produced by tissue engineering methods.

Much of the (avascular) kidney development can be reproduced in organ culture of the embryonic kidney [10]. Furthermore, the individual processes of UB and MM morphogenesis have been, in large part, separated in vitro (ex vivo) using partial or modified organ cultures [11, 12]. Key soluble factors have been initially identified using these and other in vitro models [11,

13, 14] and then confirmed in vivo in knockout animals [15, 16]. Many matrix components and integrin receptors have been identified [17, 18].

By following a developmental logic, the kidney has been reconstituted into functional tissue from these partial organ cultures (Fig. 2). This tissue is capable, for instance, of differentiated transport activity [19–22]. By microarrays, these engineered kidneys have gene expression patterns similar to an embryonic day 17–18 rat kidney (gestation being 22 days) [22, 23]. It is known that the whole embryonic kidney can be transplanted into rodents and can develop some function [24–26]. When the engineered kidney was transplanted into rodents, vascularized nephrons were observed [19]. Related to this approach, cell-based methods have had some success [22, 27]. Essentially, these studies support the view that one can begin with cells instead of primordial tissues (Fig. 2). Moreover, it is possible to dissociate the embryonic kidney into its cells and minimize

apoptosis through rho-kinase inhibition [28], and the cells can reassemble into 3D kidney-like structures [29].

Many other approaches are being applied to the tissue engineering of the kidney and other 3D epithelial organs [1, 19, 22, 29–34]. It also seems reasonable to expect that cells from various sources can eventually be differentiated to acquire characteristics of a variety of tissue-specific mesenchymes capable of stimulating branching and differentiation of primordial epithelial buds of their respective organs—similar to what the MM cells do for the UB.

Indeed, common to the development of most epithelial organs is the process of branching morphogenesis. The question arises, then, whether this developmental process—which can be thought of as the ductal or tubular branched scaffold around which the whole organ is assembled during development— can be used for a tissue engineering strategy. In other words, can the intrinsic power of branching morphogenesis critical to the development of various epithelial organs be harnessed to engineer large numbers of different branched epithelial tissues or organs?

BRANCHING MORPHOGENESIS IN THE KIDNEY AND OTHER ORGANS

Central to epithelial organogenesis of the kidney, lung, breast, prostate, salivary gland, pancreas, and other epithelial organs is the process of branching morphogenesis [35]. It has been the subject of study for many years in the field of organogenesis— using such diverse approaches as in vivo gene knockouts, whole organ culture, partial organ culture in 3D (e.g., isolated UB culture in a 3D matrix [12]), culture of epithelial cells in gels stimulated by various growth factors, and other methods. Although there are differences between various epithelial organs, in general, the developmental process begins with the formation of an epithelial bud, which then undergoes repetitions of branching to create new tips and stalks— often in a stereotypical pattern (roughly 20 rounds in the developing human kidney, for example, and several more in the developing lung).

Essentially the epithelial bud becomes an iterative tip-stalk generator (ITSG) [2–4, 36]. This generator seems to be powered by a network involving dozens of genes that, despite differing in some specifics from organ to organ (i.e., the particular set of fibroblast growth factors, transforming growth factor-β [TGF-β] superfamily members, other heparan-binding growth factors, intracellular signaling pathways, integrin receptors, heparan sulfate proteoglycans involved, and transcription factors) are remarkably— one might say, stunningly—similar when one looks at the list of branching genes implicated over the years by in vivo (i.e., knockout) and in vitro studies (such as those mentioned above) of each organ's development (Table 1).

An example might be helpful. Cell culture models of branching morphogenesis (Fig. 3) using renal adult or embryonic cell lines (i.e., MDCK, IMCD, and UB cells) indicate the importance of several growth factor signaling pathways (including those mediated by epidermal growth factor receptor ligands, hepatocyte growth factor, various TGF-βs and bone morphogenetic proteins, pleiotropin, various fibroblast growth factors, and others) in regulating branching morphogenesis in 3D extracellular matrix (ECM) gels [37, 38]. Many of the ECM proteins necessary for branching, their integrin receptors, heparan-sulfate proteoglycans, intracellular signaling pathways, and ECM-digesting proteases (e.g., matrix metalloproteinases) have been identified.

Table 1. A list of some of the various molecules implicated in the modulation of epithelial branching morphogenesis in kidney, mammary gland, and lung [6, 61–65]

Branching morphogenesis modulators	Organ system		
	Kidney	Mammary gland	Lung
Growth factors and receptors			
Fgf1	×		×
Fgf2	×	×	
Fgf7	×	×	
Fgf10	×	×	×
Hgf	×	×	×
Tgfa	×	×	
Activin	×	×	
Bmp4	×	×	×
Tgfβ	×	×	×
Gremlin1	×		×
ErbB/neuregulin/heregulin	×	×	
Fgfr2	×	×	×
Egfr	×	×	
Transcription factors			
Pax2	×	×	
Eya1	×		×
Six1	×		×
Wnt4	×	×	
Wnt5a		×	×
Wnt11	×	×	
β-Catenin	×	×	×
Pea3/Etv4	×	×	×
Extracellular matrix			
Hs2st	×	×	
MMP2	×	×	×
MT1-MMP	×	×	×
Fibronectin	×	×	×
Other molecules			
Notch	×	×	×
Sonic Hedgehog	×	×	×
Sprouty1	×		×
Sprouty2		×	×
SFRP1	×		×
Semaphorin3a	×		×
Slit/Robo	×	×	×
Eph/Ephrin	×		×

The isolated UB (the embryonic primordial tissue out of which the branched urinary collecting duct system of the kidney arises) undergoes branching morphogenesis using many of the same molecules as the epithelial cells in 3D culture (or closely related molecules) [14, 28, 39, 40]. Initially, the murine knockout data in the kidney development field suggested that some of these molecules might be less important in vivo than in vitro. But it is now becoming evident, from more careful examination of the knockouts, as well as from double knockouts, that similar sets of genes are necessary for branching of the UB in vivo. For instance, the hepatocyte growth factor (c-met receptor) was first directly implicated in branching of MDCK cells [41]. However, in other renal epithelial cell lines (UB and IMCD cells), arguably more relevant to UB and collecting duct development, epidermal growth factor (EGF) receptor ligands appeared equally important [42–44]. Nevertheless, embryonic kidney organ culture studies clearly suggested that hepatocyte growth factor was important for branching [45–47]. This led to some debate. It was subsequently shown that whereas the single knockout of the c-met does not result in a detectable phenotype, the double knockout of c-met and the EGF receptor has a branching defect [16], which was predicted from in vitro UB and IMCD cell culture studies. Furthermore, in the mammary gland, knockout of c-met alone alters branching [48]. This is but one example of the growing concordance of in vitro
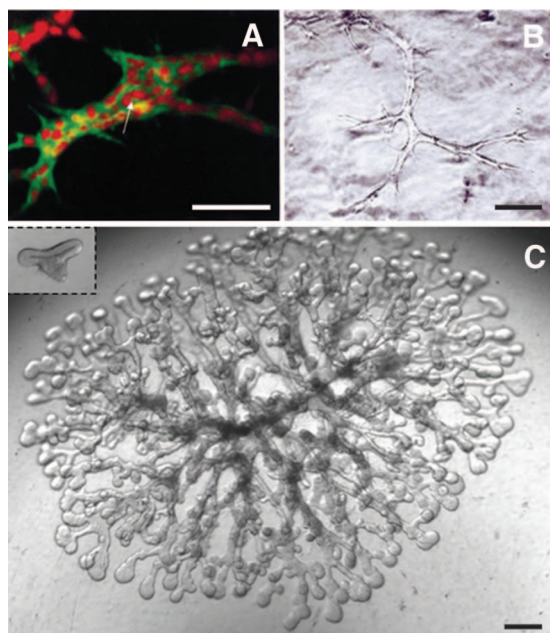
Figure 3. Branching morphogenesis of epithelial cells and isolated ureteric buds (UBs) embedded within a three-dimensional (3D) extracellular matrix (ECM) gel. **(A, B):** Photomicrographs of UB cells grown for 12 days in a 3D ECM gel composed of a 1:1 mixture of collagen (type I) and Matrigel. **(A):** Fluorescent photomicrograph of cultured UB cells that have formed multicellular-branched tubular structures with lumens (indicated by the arrow). Green, *Dolichos biflorus* lectin; orange, propidium iodine (orange). Scale bar = 40 μm. **(B):** Phase contrast photomicrograph of UB cells cultured for 12 days in 3D ECM gels. Scale bar = 40 μm. **(C):** Phase contrast photomicrographs of UBs isolated from embryonic day 13 rat kidney and cultured for up to 8 days in 3D ECM gels in the presence of a morphogenetically active fraction of BSN-conditioned media supplemented with 10% fetal calf serum, 125 ng/ml glial-derived neurotrophic factor, and 250 ng/ml fibroblast growth factor 1. Inset: Freshly isolated T-shaped UB embedded within 3D ECM gel. After 8 days of culture the UB has undergone extensive growth and branching, resulting in the formation of a highly branched structure with clear tips and stalks. Scale bar = 200 μm. Adapted from [66]; used with permission.

and in vivo data on branching morphogenesis. Although there is some variability from organ to organ, similar sets of genes are implicated in epithelial branching in a number of organs. The similarities between the kidney, lung, mammary gland, prostate, and salivary gland are particularly striking (Table 1).

In the kidney, severe branching phenotypes are uncommon. Often, the kidney retains more than half of its nephrons despite perturbation of what is thought to be a key morphogenetic pathway [49]. This may be due to several conditions operative in vivo. (a) Branching morphogenesis in the kidney (and probably other organs) is protected because of redundant pathways [2, 3, 16, 21, 36, 50]. (b) There may be a decentralized branching network with many important nodes but few central hubs such that robust branching can occur even when an important node is disrupted by bypassing that node [2–4, 36]. (c) The (metanephric) mesenchyme cells surrounding these branching structures have a remarkable instructive capacity [21]—indeed, they have been proposed to contain cells with stem-like properties [51]. Perhaps MM cells have the capacity to repair branching defects.

THE ITERATIVE TIP-STALK GENERATOR

Even though the lists of genes and pathways are quite similar in different developing organs undergoing branching morphogen-

esis, the details of the network topology may turn out to be somewhat different from organ to organ once gene interactions necessary for branching morphogenesis are worked out [3]. For example, sex steroid hormone receptor signaling is likely to be much more important in the mammary gland than in other branching epithelial organs [52].

Even so, in this era of synthetic biology—and in the context of what is known about branching morphogenesis in various epithelial organs (from in vitro studies in particular)—it seems reasonable to conceive of a universal epithelial ITSG, powered by a branching gene network, around which epithelial organs may be built. How such a branching network might power the ITSG is a very intriguing question to explore.

Continuing to use the kidney as an example, let us consider the UB, which can be cultured in isolation within a 3D ECM gel [12] (Fig. 3). In the presence of a particular growth factor cocktail (consisting of soluble factors derived from the MM), the UB becomes an impressive ITSG in vitro. A major point to keep in mind again is that the growth factors and matrix-related molecules involved in UB branching, not to mention other branching genes, are very similar to those molecules critical for branching morphogenesis in the developing mammary gland, lung, and other epithelial organs (Table 1). This is all the more remarkable because of the very distinct roles that these organs, when fully differentiated, play in systemic physiology. Consider, for instance, the different physiological functions of the kidney, lung, and mammary gland. It is also interesting to note that although most are cases of predominantly prenatal development through branching (e.g., kidney, lung, pancreas), others (e.g., mammary gland, prostate) are cases of predominantly postnatal development and maturation.

Then where would organ specificity come from? The answer, possibly, is that it may in large part come from the mesenchyme. This has major implications for the tissue engineering of complex 3D epithelial organs. Unlike the kidney, where the MM actually becomes part of the structural and functional epithelial unit (the nephron)—after itself transforming into epithelia via a mesenchymal-to-epithelial transition—in general, the mesenchyme of these other developing organs does not appreciably epithelialize and become incorporated into the functional ductal epithelial units of epithelial organs such as the mammary gland and lung (ultimately capable of producing milk proteins and surfactant proteins, respectively). Instead, the mesenchyme plays a key instructive role, and this may be a general rule, even for the kidney MM (despite the fact that it epithelializes to form the structural proximal parts of the nephron and glomerulus) [21].

Of particular interest in this regard are recombination experiments between branching epithelial structures of various organs with the mesenchyme of a different organ. When the branching UB of the developing kidney is recombined in vitro with lung mesenchyme, the kidney-derived branching epithelial cells produce surfactant protein [53]. When salivary gland mesenchyme is recombined with mammary ductal tissue, the mammary ducts branch with a salivary gland pattern [54].

What this suggests is the following. The universal ITSG—something of a theoretical construct at the moment [2–4], but nonetheless consistent with work in many systems—would have the potential to be recombined with organ-specific mesenchyme, or mesenchymal cells differentiated toward that organ, to build the whole organ; the type of organ would be determined by the kind of mesenchyme used in the recombination. Importantly, our universal
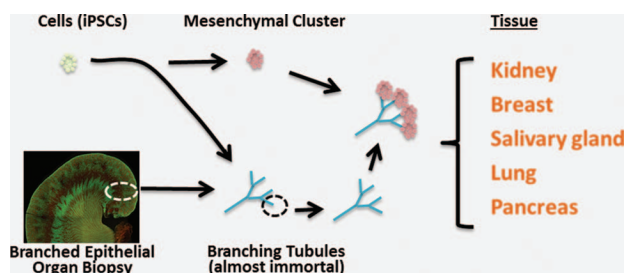
Figure 4. Schematic approach for engineering of different organ-like tissues. Figure shows the putative approach for engineering epithelial organ-like tissues using either induced pluripotent stem cells or cells derived from a biopsy of a branched epithelial organ (i.e., kidney), which are induced to form a branched tubular structure (i.e., iterative tip-stalk generator). The branched in vitro-formed tubule is then recombined with a cluster of organ-specific mesenchymal cells of various tissues or cells (e.g., mesenchymal stem cells, iPSCs, or other cells) that have been differentiated toward organ-specific mesenchyme. After days or weeks of mutual induction, the recombined tissue resembles the tissue specified by the organ-specific mesenchyme cells (e.g., kidney, lung, pancreas, salivary gland, mammary gland). Abbreviation: iPSC, induced pluripotent stem cell.

ITSG would form a three-dimensional branching tree that would serve as a 3D cellular scaffold, establishing the architecture of the engineered tissue even as it continues to grow, branch, differentiate, and acquire organ-specific characteristics.

So how might one generate the universal ITSG? There are some hints. The aforementioned data from various branching epithelial organs raise the possibility that some developing branching tissues, when cultured in isolation as described above for the isolated UB culture, have a partial ITSG character. It will be interesting to see to what extent they are interchangeable in the types of recombination experiments described above [53]; much more needs to be done in this regard. Moreover, most in vitro experimentation with these culture systems has been aimed at mimicking the parent organ; for example, nearly all work with the isolated UB culture system has been focused on creating conditions as faithful as possible to in vivo UB branching (in the context of the MM) occurring during kidney development. What if the goal were to construct an ITSG out of the UB? The work has not yet focused on trying to turn the isolated UB culture or isolated lung bud culture into something with properties of a generic ITSG; by altering culture conditions, it may be possible to head in this direction.

Now, suppose a best attempt at making an ITSG, and also suppose different organ-specific mesenchymes (or cells that have been differentiated toward organ-specific mesenchyme). What can be done with these? One hope is that we might recombine as required to produce different epithelial organs from our ITSG (Fig. 4). We already know that, even for an organ as complex as the kidney, we can start with the isolated cultured branching UB (indeed, even with the isolated cultured Wolffian duct before it becomes a UB) and recombine with the MM to obtain tissue with functional nephrons that can be implanted in rodents and get vascularized [19, 22]. So, perhaps this can be done for lung, pancreas, salivary gland, mammary gland, and so on as well.

The considerable work using various sources of stem cells and mesenchymal cells to differentiate them down particular organ-specific cell lineages suggests that it is not unrealistic to expect in the future an abundant and reliable source of organ-specific mesenchyme-derived cells [55]. If they are derived from mesenchymal stem cells or induced pluripotent stem cells, these organ-specific mesenchyme cells could also be patient-specific.

How can a similar abundant source of ITSGs be obtained? Bear in mind that the ITSG is a dynamic 3D tree. Its branching tips not only can induce mesenchyme but also can incorporate into the functional units (e.g., nephrons) as described above [19, 21]; the ITSG will be the 3D branching cellular scaffold around which the organ is built. But how can we make many ITSGs without starting anew with a microdissected embryonic structure each time? The answer is that we take a "gardener's approach" to the ITSG that is being cultured [20]. Metaphorically, one might imagine taking clippings of branches and planting them separately. In other words, one could microdissect and subculture tips (or tips and stalks) in new 3D matrices with the appropriate growth factors and supplements (within some sort of bioreactor) [30]. And when these ITSGs branch sufficiently, the process can be repeated. Thus, by taking advantage of the intrinsic power of the branching process (presumably this power derives from the network of interacting genes responsible for branching morphogenesis), a single ITSG serves as an abundant source of other ITSGs [30].

Something like this has been done with the isolated UB culture in the context of the developing kidney [19, 20, 30]. When grown sufficiently in 3D culture, portions containing tips and stalks were microdissected and then recultured (Fig. 5). The recultured isolated UBs, when grown into larger branched structures, were then recombined with MM to yield kidney organoids that appeared similar to the embryonic kidney in whole organ culture [30]. The MM-derived nephron connected to the UB-derived branching collecting ducts (Fig. 6). This process was repeated through another generation. Whether this can be repeated for a very large number of generations is unknown. Nevertheless, it remains that a single UB yielded many new UB-like structures, and when these new UB-like structures were recombined with MM, they became kidney organoids reminiscent of the embryonic kidney itself with, at least superficially, the appropriate tubular "plumbing" (i.e., connections between the distal tubule and the collecting duct). The extent to which cells capable of forming a microvasculature can be induced to branch alongside the 3D cellular scaffold of the ITSG—in an in vitro culture system such as the isolated UB— has not been well explored. Branching of the UB tends to be studied independently of branching of the vasculature. It is worth noting, however, that overlapping or similar sets of growth factors and ECM molecules play key roles in branching of epithelial cells and endothelial cells [56–58].

Although most of branching morphogenesis occurs during embryonic development of organs, in some cases, such as the mammary and prostate glands, it can occur after birth. If, for the purpose of obtaining a patient-specific ITSG, it is important to begin with ductal cells that have the ability to branch after birth, these are relatively accessible tissues. But it may be possible to use mature cells; some of the animal cell lines that have been quite useful in studying branching morphogenesis (e.g., IMCD cells) are derived from adult organs [42]. This leaves open the possibility of trying to generate a patient-specific ITSG from, for example, an adult salivary gland biopsy. The salivary gland contains a progenitor cell population capable of forming apparent branched epithelial structures reminiscent of the branching UB in isolated culture [59]. And there is also the possibility, if protocols can be worked out, of using patient-derived stem cells as a starting point for the ITSG.

Of course, a great deal more work needs to be done with the latest expression profiling techniques to determine the extent to which organ-specific mesenchymal cells can program the ITSG
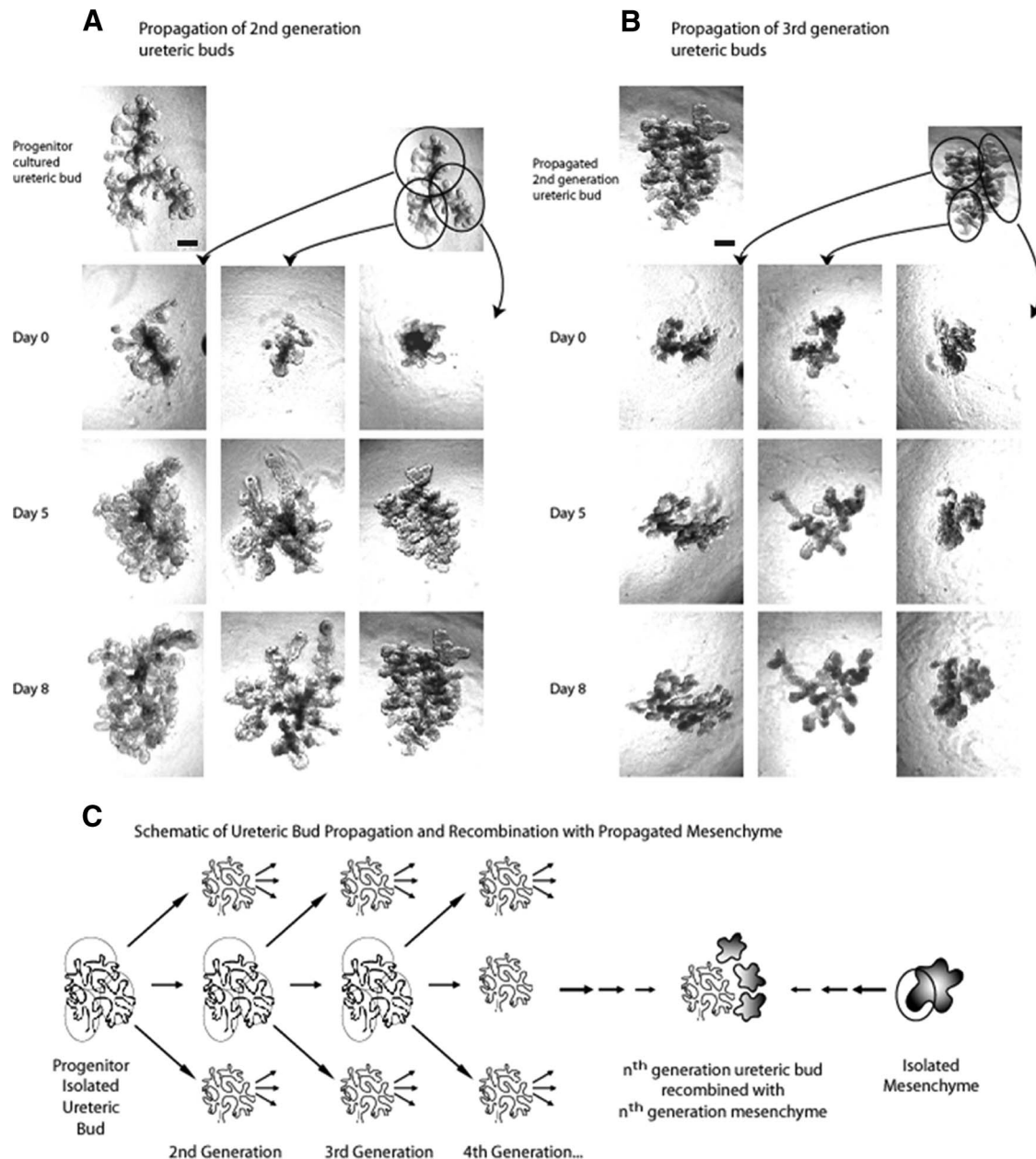
Figure 5. Ex vivo propagation of isolated ureteric buds (UBs). **(A, B):** Phase contrast photomicrographs of isolated UBs cultured in 3D extracellular matrix (ECM) gels in the presence of BSN-conditioned media supplemented with 10% fetal calf serum, 125 ng/ml glial-derived neurotrophic factor, and 250 ng/ml fibroblast growth factor 1. **(A):** After 8 days of culture a UB was subdivided into thirds and recultured in new 3D ECM gels with the fresh media (day 0). Over the course of the next 8 days, these second-generation UBs proliferated and branched in a manner similar to the progenitor UB. Scale bar = 100 μm for all images. **(B):** A second-generation UB was subsequently subdivided into thirds and recultured within ECM gels for an additional 8 days. Growth and branching was evident, but to a lesser extent than that seen in the original subdivsion. Scale bar = 100 μm for all images. **(C):** A schematic representation of UB propagation demonstrates a potential colony with a large number of UBs derived from a single progenitor bud, which can then be recombined with separately propagated mesenchyme. From [30]; used with permission.

toward organ-specific ductal functions. Given that, in organs such as the kidney, a mutual inductive process between the branching ductal system and mesenchyme is essential to organogenesis, it is also conceivable that propagated organ-specific ITSGs in 3D cultures (e.g., isolated UB) can be recombined with undifferentiated mesenchymal stem cells to generate 3D organs. Again, much more work needs to be done with specific markers and the latest expression profiling techniques to assess the feasibility of these ideas in the context of tissue en-

gineering of each epithelial organ. Certain organs may prove more amenable than others.

OTHER CONSIDERATIONS

Whether a universal epithelial ITSG can be devised with sufficient flexibility to differentiate into the branched ducts or tubules of organs as functionally distinct as the kidney, lung, pancreas, salivary gland, breast, prostate, thyroid, biliary tract, and
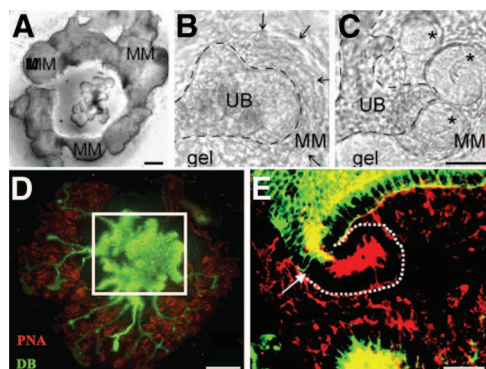
Figure 6. Recombination of cultured ureteric bud (UB) and freshly isolated MM. **(A–C):** Phase-contrast photomicrographs of uninduced MM placed around a cultured UB. The clear area between the UB and MM is the residual extracellular matrix gel in which the UB was cultured **(A)**. Scale bar = 200 μm **(A)**. **(B, C):** After 8 days of coculture cellular interactions between an ex vivo-cultured UB and isolated MM were evident. Scale bar = 50 μm. **(D, E):** Fluorescent confocal images indicating interactions between the UB (green, *Dolichos biflorus*) and MM (red, E-cadherin) recombinations after 8 days of coculture. Scale bar = 200 μm **(D)**. **(E):** MM-derived epithelial tubular structures were observed (red structure outlined by white dashed line; green is cultured UB). Arrow: Connection of the UB with the MM. Scale bar = 100 μm. Adapted from [21]; used with permission. Abbreviations: DB, *Dolichos biflorus*; MM, metanephric mesenchyme; PNA, peanut agglutinin; UB, ureteric bud.

so on is far from clear. These distinct functions arise, among other things, as a result of the appropriate expression of tissue-specific sets of transporters and channels at the apical and basolateral surfaces of the polarized epithelial cells lining the ducts and tubules of different epithelial organs [60]. For example, the proximal tubule of the kidney must be capable of vectorial transport (usually plasma to urine) of drugs and toxins, and the collecting ducts must be capable of concentrating the urine by absorbing large amounts of water [7]. The regulation of these and other tissue-specific genes is governed by particular sets of transcription factors that may need to be activated in the proper spatiotemporal contexts [55]. Also unclear is whether organ-specific mesenchyme cells alone are sufficient to induce the ITSG to adopt the functional tissue-specific properties of mature ducts and tubules. Certainly, much will depend on technical issues not discussed here: cell sources, matrices, dimensionality, bioreactors, and exogenous agents (e.g., small molecules, growth factors) that might facilitate differentiation toward one organ or another.

To the extent that such engineered organ-like constructs prove useful for tissue toxicity studies, developmental biology, and physiology, they may help minimize the need for in vivo animal studies. Of course, one of the main goals in the field tissue engineering is to provide organ-like tissues suitable for incorporation into medical devices, for transplantation or for other regenerative medicine purposes. But it is important to keep in mind the clinical situation in which the use of an engineered tissue might be contemplated. Going back to the example of the kidney, renal insufficiency is frequently associated with chronic systemic diseases (e.g., diabetes, hypertension, systemic lupus erythematosis), and there are circulating uremic toxins that affect cell function. A tissue engineered organ or organoid must survive and adapt in such a setting while it takes on normal kidney function. Thus, recapitulating structure and some significant functions may not be enough. The engineered kidney must also be able to respond to the various acute and chronic stresses that the normal kidney is routinely exposed to, including volume depletion, ischemia, exposure to drugs, and environmental toxins. That is asking a lot of a tissue-engineered construct, but there seems to be no obvious barrier to achieving this capability over the long term. Indeed, the apparent plasticity of developing tissue may be advantageous here; such tissue may have greater ability to adapt in the diseased setting. But this, as with much of what has been discussed above, remains to be demonstrated experimentally. What we have right now are tantalizing hints that it may be possible to engineer large numbers of different epithelial organs by taking advantage of the power of branching morphogenesis and combining it with instructive skills of tissue-specific mesenchyme cells.

AUTHOR CONTRIBUTIONS

S.K.N.: manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

S.K.N. has an uncompensated interest as an inventor on a patent and/or application related to some aspects of what is discussed here.

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